MICROBIAL ACTIVITY IN A PODZOL

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Introduction

Over recent years some confusion has arisen over the term 'activity', as applied to soil micro-organisms, and to its assessment. This confusion has been particularly noticeable in considerations of the fungal component of soil populations, where, until recently, estimates of activity have been almost solely based on soil dilution plate counts — a technique which in reality only allows estimation of the sporing capacity of the soil fungi. Recently attempts have been made to assess fungal activity on the amount of hyphae present in soil (as indicated by direct observation techniques or by the use of isolation methods which allow the selective isolation of fungi present in the soil as hyphae), the argument being that it is in the hyphal state that fungi are physiologically active. However such assessments, based on the amount of microbial material present, may be misleading — fungal hyphae observed or isolated may have been in a resting state or metabolizing slowly in the undisturbed soil.

Thus, if we are concerned with the measurement of physiological activity of soil populations, some other method or methods must be used. Probably the most widely used method for assessing the biological activity in soil is the measurement of oxygen uptake by soil samples, and the use of this data as an index of activity. From the early part of this century (RUSSELL, 1905) such measurements have been made, but these measurements were made with large soil samples over quite a long period of time (at least several days). More recently, following exploratory work (WEBLEY, 1947; QUASTEL and WEBLEY, 1947; ELLINGER and QUASTEL, 1948), ROVIRA (1953) suggested that the Warburg respirometer could be used to measure the respiratory activity of soil, and that it had advantages in that oxygen uptake could be accurately measured over short periods of time. Since this initial application of the Warburg technique, numerous workers have applied it in soil microbiological investigations, and many of these studies are reviewed by CHASE (1958).

The development of macro-respirometers (SWABY and PASSEY, 1953; WIERINGA and KERKHOF MOGOT, 1956) have allowed accurate studies to be made on large soil samples over long periods of time, and the development of manometric percolation techniques (GREENWOOD and LEES, 1959) has provided elegant means for the study of various biochemical processes in soil and for the estimation of the metabolic potential of soil (which must be an important consideration to the microbiologist working with agricultural or forest soils).

In the study of the microflora of the iron-humus podzol at Delamere, Cheshire, measurements of microbial activity (as given by oxygen uptake measurements) have been made, supplementing the data obtained from the isolation and direct observation of the fungal component of the microflora of this soil. In the activity measurement, which are presented here, no attempt has been made to elucidate the relative roles of bacteria, fungi, actinomycetes, mites etc., and the measurements presented represent the total biological activity of the different horizons of this podzol.

Methods

Ten soil samples from each horizon of the Delamere podzol (L, F_1, F_2, H, A_1, A_2, B_1, B_2 and C horizons) were taken from one large pit. These samples were brought to the laboratory and stored overnight at 2° C. The ten samples from each horizon were then mixed; the L, F_1 and F_2 material was sorted to prevent, as far as possible, the inclusion of twigs and bark in the experimental samples, and the samples of H—C horizon soil were sieved, the material passing through a 2 mm sieve being used in the subsequent oxygen uptake measurements.

5—35 g sub-samples of soil from each of the horizons F_2 to C were then weighed into each of three of the specially constructed respiration flasks (Fig. 1), and sub-samples from each horizon were taken for determinations of moisture content and organic matter content.

Material from the L and F_1 horizons was placed in special flasks (described by JOHNSTON, 1953) in which the pine needles, the predominant organic matter source in this soil, could be placed without cutting.

In all flasks 10 % KOH was present to absorb the CO_2 evolved during respiration.

The flasks were attached to the manometers and placed in the water bath, here the soil was allowed to settle for 20 hours, during this period the mano-

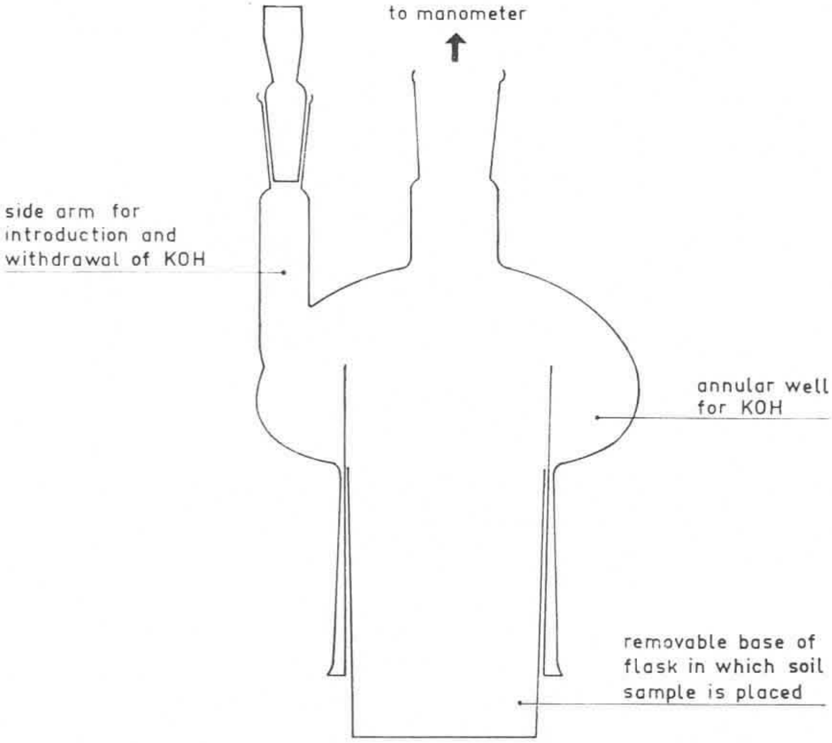
to manometer
side arm for introduction and withdrawal of KOH
annular well for KOH
removable base of flask in which soil sample is placed

Fig. 1. Diagrammatic section of a respiration flask used for measurements of O_2 uptake of F_2—C horizon material.

meter tap was open. After this settling period, which was at room temperature, and after an equilibration period of one hour at the temperature at which soil respiration was to be measured (i.e. 25° C), the manometer tap was closed and measurements of oxygen uptake by the soil samples were begun, hourly readings of oxygen uptake over a 5 hour period being made.

Thus the technique used was one of direct measurement of oxygen uptake (UMBREIT, BURRIS and STAUFFER, 1957) of fresh soil — not on air-dried, re-moistened material.

Results and Discussion

The data obtained on the respiratory activity of the different horizons of the Delamere podzol are presented in Table 1 (this data being based on soil samples taken in October 1961). The values shown in Table 1 relate to unit weights of oven dry soil (the soil being dried to constant weight at 105° C). From this data it can be seen that the respiratory activity is much greater in the A_0 complex than in the mineral horizons of the podzol.

The sub-divisions of the A_0 horizon (the L, F and H layers) obviously contain microfloras of different activity — as indicated by the oxygen uptake

TABLE 1. Oxygen uptake of soil from the different podzol horizons (Measurements based on unit oven dry weight of soil)

Horizon	Oxygen uptake ($\mu l\ O_2$/5 hr/g oven dry soil)
A_0 L	2366.0
F_1	1400.0
F_2	245.2
H	80.9
A_1	13.3
A_2	4.5
B_1	9.8
B_2	2.9
C	1.4

data — with the L layer as that which supports by far the most active microflora. The sub-division of the F layer into F_1 and F_2, which was shown to be necessary on morphological grounds (KUBIENA, 1953) and on microbiological grounds (KENDRICK, 1958) is also shown to be supported on the grounds of microbial activity in the two sub-divisions, i.e.

F_1 layer	F_2 layer
Needles entire but black	Needles fragmented
Dominant fungi: *Desmazierella* *Helicoma* *Sympodiella*	Dominant fungi: *Trichoderma* *Penicillium* *Mucor*
Greater microbial activity	Lesser microbial activity

In the mineral horizons it appears, from the data in Table 1, that the A_1 and B_1 horizons (i.e. those mineral horizons with the highest organic matter content) have the greatest activity.

It was considered, because of the great variations in organic matter content through the podzol profile (98.5 % in the L horizon, 1.4 % in the C horizon), necessary to calculate oxygen uptake in the different horizons on the basis of available respiratory material, and to do this in a simple way the oxygen uptake data was recalculated on the basis of unit weight of organic matter. This data is presented in Table 2. From this it can be seen that:—

1. Once again the L, F_1 and F_2 layers of the A_0 horizon have the highest respiratory activity, the relative activity in these layers being as in the previous calculation (Table 1).

TABLE 2. Oxygen uptake of soil from the different podzol horizons (measurements based on unit weight of organic matter)

Horizon	% organic matter	Oxygen uptake (μl O_2/5 hr/g organic matter)
A_0 L	98.47	2406.0
F_1	98.06	1428.0
F_2	89.30	274.6
H	54.58	148.3
A_1	17.16	77.7
A_2	1.87	238.8
B_1	10.62	91.9
B_2	5.24	56.6
C	1.42	96.3

2. The H layer shows an 85 % increase in activity (80 μ l/g soil —148 μ l/g organic matter), but this still indicates that the A_2 horizon has greater activity per unit weight of organic matter.
3. Of the mineral horizons, the A_2 horizon emerges with a rate of oxygen uptake higher than that of any other mineral horizon and higher than the H layer.
4. The C horizon shows the greatest increase in activity when calculations are made on unit weight of organic matter as opposed to unit weight of oven dry soil.

Thus the order of activity in the horizons, based on oven dry soil basis, of:

$L > F_1 > F_2 > H > A_1 > B_1 > A_2 > B_2 > C$

gives way, on unit weight, of organic matter basis, to:

$L > F_1 > F_2 > A_2 > H > C > B_1 > A_1 > B_2$

It seems, therefore, that in the A_2 and C horizons — horizons low in organic matter, where the organic matter is present mainly as sparsely distributed macroscopic fragments (not as amorphous material coating mineral grains) — microbial activity, where it is possible (i.e. based on the organic fragments), is more vigorous than in the other mineral horizons. It is not possible, at the moment, to explain these observations. It may be that in the A_1 and B_1 horizons there is too much organic matter for the micro-organisms present, or it may be that water soluble inhibitors are having an effect (particularly in the A_1 horizon), whilst in the B_1 horizon a good deal of the organic matter comprises humic material which is not readily decomposed by many fungi.

The B_2 horizon would seem to be an horizon which is relatively uncongenial for microbial development and activity.

The data given above are based, as described in the Methods section, on ten soil samples from each horizon at one site at Delamere. In an attempt to assess spatial variations in the microbial activity in particular horizons at one sampling time, soil samples from an horizon were taken from each of a number of sites in the Delamere area in February 1961. The oxygen uptake measurements were made, as previously described, on the soil taken from each sampling site. Table 3 gives data on the variation in oxygen uptake, organic matter content and moisture content of samples from the A_1 horizon taken from 7 sites. From these data it can be seen that considerable variation in oxygen uptake was recorded from the different sites (from 3.8 to 23.8 μl/5 hr/g dry wt. soil, or 59.4 — 158.7 μl/5 hr/g organic matter). In considering the reasons for this variation it appears that soil moisture content is an operating factor. In considering the oxygen uptake data based on unit weight of organic matter, this increases with increasing soil moisture content up to 22.1 % after which activity appears to fall off. Correlation of soil moisture content with oxygen uptake has been noted by other workers, who varied the moisture content of soils whilst studying their respiratory rates (GAARDER, 1957), and DOUGLAS and TEDROW (1959) using respirometric techniques showed organic matter decomposition to be highly influenced by temperature and moisture content.

TABLE 3. Variation in oxygen uptake of soil from the A_1 horizon sampled from several sites at the same sampling time (results expressed as μl O_2 per gram organic matter)

Sample No.	% moisture content	% organic matter	Oxygen uptake (μl O_2/5 hr/g organic matter)	Oxygen uptake (μl O_2/5 hr/g oven dry soil)
1	11.5	6.48	59.4	3.8
2	15.1	7.11	88.5	6.3
3	18.9	9.25	95.3	8.8
4	20.0	10.16	101.3	10.3
5	22.1	11.39	158.7	18.1
6	23.9	18.00	132.1	23.8
7	31.0	16.00	116.7	18.7

Preliminary results, obtained in September 1959, indicated the importance of moisture content in affecting microbial activity in the A_0 horizon. Table 4 gives data on oxygen uptake measurements taken on a series of samples

TABLE 4. Oxygen uptake of L layer material at different natural moisture contents

% moisture content	Oxygen uptake (μl O_2/hr/g oven dry wt.)
14	13
25	99
28	99
49	357
50	355
59	390
61	400

from the L layer. This sampling was begun at the end of a six week dry period and was continued for some time after the normal rainfall pattern had been resumed. Therefore this data is made more complex by the fact that the different samples recorded were subject to different field temperature conditions (although respiratory activity was measured at the standard 25° C). However the data serve to indicate the possible effect of moisture content on microbial activity in the freshly fallen litter — a layer which is particularly susceptible to climatic changes.

It is appreciated that the data which are presented do not allow calculation of the actual microbial activity in the natural undisturbed soil horizons. However the values obtained, accurate comparative data on potential activity under defined experimental conditions, outweigh this disadvantage. The technique may be extended in various ways — selective inhibitors may be used to elucidate the relative roles of the components of the soil microflora, natural substrates may be added to the soil to study the potential biochemical activity of the soil microflora, and environmental conditions (temperature, moisture content, carbon dioxide concentration etc.) may be experimentally altered and the effects of these alterations assessed. Thus oxygen uptake measurements (using micro-respirometric methods) allow a more precise and meaningful approach to the problem of the activity of the soil microflora.

Summary

The use of oxygen uptake measurements in assessing the microbial activity in the organic and mineral horizons of an iron-humus podzol is described. The use of modified respiration flasks gives data indicating much higher microbial activity in the L and F layers than in the other horizons, and substantiates the sub-division of the F layer into F_1 and F_2 layers. Data is presented which indicates that microbial activity in a single horizon is subject to considerable spatial variation.

The effect of natural soil moisture content on microbial activity is discussed, and data is presented which indicates that this factor is important in governing microbial activity in soil.

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Discussion

E. G. MULDER: In Warburg experiments with soil we found a considerably higher uptake when using CO_2 buffer than with KOH for absorbing carbon dioxide. This was true when using acid soil but not with neutral soil. Our experiments were continued over some days; this may have been the difference with Dr. Parkinson's experiments which lasted only 5 hours. It is a well known fact that CO_2 is required by heterotrophic microorganisms. We found it to be essential for the formation of nodules in leguminous plants and for the process of nitrogen fixation in nodules. The same was found to be true of freeliving nitrogen fixing bacteria as *Beijerinckia*. The difficulty in showing a CO_2 requirement is to remove the CO_2 formed by the micro-organisms. Therefore a culture medium with a low pH is much more convenient than a neutral medium because there is practically no bicarbonate present.

D. PARKINSON: The soils had a pH 3.5—4.5 and showed no difference in oxygen uptake in relation to varying CO_2 concentrations.

C. A. EDWARDS: What proportion of the respiration is due to animals.

D. PARKINSON: This is a most important question, and one which is extremely difficult to elucidate. The use of selective inhibitors such as aldrin may help, but such inhibitors must be used with care, and examination of which organisms are killed must be made. There is also the possibility that a chemical may inhibit some organisms but be used as a substrate by others.

C. A. EDWARDS: Microorganisms can in fact use aldrin as a substrate in breaking it down by oxidation into another insecticidally active material dieldrin.

H. L. JENSEN: Aldrin may serve as a substrate to certain microorganisms, but its breakdown is a very slow process unlikely to appear in Dr. Parkinson's short term experiments. It is possible that it could have an inhibiting effect on some components of the soil microflora.

G. W. HARMSEN: Is it an adaptive enzymatic action, and therefore without influence in short term experiments.

H. L. JENSEN: I have not personally studied the breakdown of aldrin but am quoting Dr. Fåhraeus of Uppsala. Its breakdown would almost certainly be due to inducible enzymes.

P. BERTHET: Quelques tentatives faites dans le domaine, dans notre département, ont montré que pour les couches superficielles de la litières des forêts, les variations du consommation d'oxygène d'un échantillon à l'autre pris au même endroit et au même moment étaient considérables et rendaient difficiles toute comparaison entre des échantillons provenant de differents milieux ou de differentes périodes. Quel est l'ordre de grandeur des variations d'un échantillon à l'autre observé par Dr. Parkinson.

D. PARKINSON: Disturbance of the soil causes an increase in oxygen uptake which then falls and at 20 hours reaches a steady state. Variation in the L layer was about 10 % (2000—2600 μl 0_2/5 hr/g). In mineral horizons 25 gram samples gave less variation than 2—3 gr. samples. Cut needles gave higher respiratory activity than whole needles.

C. H. DICKINSON: Have you information on the temperature and watercontent of the various profiles?

D. PARKINSON: No.

C. H. DICKINSON: Have you information on the soil atmosphere in the different horizons and what is the depth of these.

D. PARKINSON: Field estimations of the oxygen carbon dioxide tensions are difficult to obtain and so laboratory experiments at 25° C were used. Temperature has a marked effect but moisture is the most important factor. The depth of the different horizons is variable.

C. H. DICKINSON: If temperature and other factors vary in different layers of the profile than correction curves similar to that of O'Connors (fig. 46) may have to be applied and this may make results from different layers more similar.

A. BURGES: Carbon dioxide concentrations in the soil can vary from that in air to $> 10\%$ following rain. These fluctuations can occur very rapidly therefore occasional measurements are of little value. Even if one can measure the level of CO_2 continually it is still difficult to know how to use this information.

G. W. HEATH: I would like to support Professor Burges in his criticism of the value of spot readings of CO_2 concentration in situ (in the soil) and to say that it has been further suggested that any true value is almost impossible to obtain because of the differential solubility of CO_2 in soil water at different CO_2 concentrations and at different quantities of soil water.